ATP 染色实验报告

一、实验器材及试剂

1、 实验器材

名称	厂家	型号
冰冻切片机	Thermo	CRYOSTAR NX50
切片刀	上海徕卡仪器有限公司	LEICA 819
防脱载玻片	Wanwu	G6004
正置光学显微镜	日本尼康	NIKON ECLIPSE E100
成像系统	日本尼康	NIKON DS-U3

2、 主要实验试剂

试剂名称	厂家	货号
OCT 包埋剂	Wanwu	G6059-110ML
Tris 碱	Sigma	V900483
氯化钙	国药集团化学试剂有限公司	10005860
ATP 钠盐	Solarbio	615B025
盐酸	国药集团化学试剂有限公司	10011008
硝酸钴	国药集团化学试剂有限公司	10007316
硫化铵	国药集团化学试剂有限公司	81001460
二甲苯	国药集团化学试剂有限公司	10023418
无水乙醇	国药集团化学试剂有限公司	100092683

二、染液配制：

2%$CaCl_2$ 溶液：5g 无水 $CaCl_2$+250ml 超纯水

2%硝酸钴溶液：5g 硝酸钴+250ml 超纯水

母液配制：Tris 碱 5g+氯化钙 0.499g+蒸馏水定容至 250ml

0.1mol/L HCL：1ml HCL+11ml 超纯水混匀后稀释 10 倍

PH10.4 孵育液：取 20ml 母液，用巴氏吸管取 0.1mol/L 的 HCL 3 滴加入，混匀，PH 约为 10.4

PH9.4 孵育液：取 20ml 母液加入 30mg 的 ATP 钠盐，混匀，用巴氏吸管取 0.1mol/L 的 HCL 25 滴加入，混匀，PH 约为 9.4。

三、染色步骤：

1、未经固定的新鲜组织冰冻切片自然晾干，组化笔画圈圈住组织，滴加 PH10.4 的孵育液孵

育 5min，倾去孵育液，不水洗直接滴加 PH 9.4 的孵育液孵育 30 min，室温下进行并加盖。

2、倾去孵育液，切片直接依次入 3 缸 $CaCL_2$ 溶液浸染，每次 2min。

3、切片直接入硝酸钴溶液浸染 5min，5 缸自来水浸洗，每缸 20s。

4、现配 1%硫化铵溶液(先取 5ml 纯水，再用巴氏吸管取 1 滴浓的硫化铵溶液加入，混匀即可，配好后立即使用)滴染或浸染 30s，3 缸自来水浸洗，每缸 20s。

5、切片依次入无水乙醇 I 5min -无水乙醇 II 5min-无水乙醇III5min -二甲苯透明 5min ，中性树胶封片。

6、显微镜镜检，图像采集分析。

四、结果判读：

I型呈浅灰色或无色，II型呈深灰或黑色，分型清楚。

五、注意事项：

1、切片一定要是新鲜组织的冰冻切片，不可以固定。

2、Tris碱、氯化钙粉剂常温保存，硫化铵常温避光保存，ATP钠盐-20℃保存，其它染液4℃保存，使用前先复常温。

3、工作液和1%硫化铵溶液要现配现用，不能保存。

ATP Staining Experiment Report

I. Experimental equipment and reagents

1. Experimental equipment

Equipment name	Manufacturer	Model No.
Freezing microtome	Thermo	CRYOSTAR NX50
Slicing knife	Shanghai Leica Instrument Co., Ltd.	LEICA 819
Adhesion microscope slides	Wanwu	G6004
Upright electron microscope	Japan NIKON	NIKON ECLIPSE E100
Imaging system	Japan NIKON	NIKON DS-U3

2. Main experimental reagents

Reagent name	Manufacturer	Item No.
OCT Embedding medium	Wanwu	G6059-110ML
Tris alkali	Sigma	V900483
CaCl2	Sinopharm Chemical Reagent Co., Ltd.	10005860
ATP Sodium salt	Solarbio	615B025
Hydrochloric acid	Sinopharm Chemical Reagent Co., Ltd.	10011008
Cobalt nitrate	Sinopharm Chemical Reagent Co., Ltd.	10007316
Ammonium sulfide	Sinopharm Chemical Reagent Co., Ltd.	81001460
Xylene	Sinopharm Chemical Reagent Co., Ltd.	10023418
Anhydrous ethanol	Sinopharm Chemical Reagent Co., Ltd.	100092683

II. Dyeing solution preparation:

2%CaCl2 solution: 5g Anhydrous CaCl2+250ml ultra-pure water.

2% Cobalt nitrate solution: 5g Cobalt nitrate +250ml ultra-pure water.

Mother liquor preparation: Tris alkali 5g+CaCl2 0.499g+distilled water, make to volume 250ml.

0.1mol/L HCL: 1ml HCL+11ml ultra-pure water, mix well and dilute for 10 times.

PH10.4 Incubation medium: Take 20mL mother liquor, add into 3 drops of 0.1mol/L of HCL with pasteur pipette, then mix well, the PH is about 10.4.

PH9.4 Incubation medium: Take 20mL mother liquor, add into 30mg ATP Sodium salt, mix well, then add into 25 drops of 0.1mol/L HCL with pasteur pipette, mix well, the PH is about 9.4.

III.Staining steps:

1. Let the frozen sections of fresh tissue without fixation dry naturally, circle the tissue with group with histochemical pen, drop PH10.4 incubation solution and incubate for 5min, tilt to pure off the incubation solution, then drop PH 9.4 incubation solution on without washing and incubate for 30min at room temperature and with cover.
2. Tilt the sections to pure off the incubation solution, and directly soak the section into 3 jars of CaCL2 solutions for dyeing in sequence, 2min each.
3. Immerse the sections directly in cobalt nitrate solution for 5min, then soak to clean in 5 tanks of tap water, 20s for each tank.
4. Drip dyeing or soak dyeing with present prepared 1% ammonium sulphide solution (Firstly take 5ml pure water, then take 1 drop of concentrated ammonium sulphide solution with pasteur pipette to add in, mix well, use immediately after it's prepared well) for 30s, then soak to clean in 3 tanks of tap water, 20s for each tank.
5. Put the sections in order into the anhydrous ethanol I for 5 min - the anhydrous ethanol II for 5 min - the anhydrous ethanol III for 5 min - Xylene vitrification for 5 min, lastly seal the sections with neutral rubber.
6. Microscope inspection, image acquisition and analysis.

IV. Interpretation of results:

Model I is light gray or colorless, model II is dark gray or black, the model classification is clear.

V. Precautions:

1. The sections must be frozen sections of fresh tissue and cannot be fixed.
2. Tris alkali and CaCl2 powder should be stored at room temperature, ammonium sulfide is stored at room temperature and protected from light, ATP sodium salt should be stored at -20℃, and other dyes should be stored at 4℃, return to normal temperature before use.
3. The working solution and the 1% ammonium sulfide solution should be prepared freshly and use immediately, and cannot be stored.